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Biobased organic acids production by metabolically engineered microorganisms

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Bio-based production of organic acids via microbial fermentation has been traditionally used in food industry. With the recent desire to develop more sustainable bioprocesses for production of fuels, chemicals and materials, the market for microbial production of organic acids has been further expanded as organic acids constitute a key group among top building block chemicals that can be produced from renewable resources. Here we review the current status for production of citric acid and lactic acid, and we highlight the use of modern metabolic engineering technologies to develop high performance microbes for production of succinic acid and 3-hydroxypropionic acid. Also, the key limitations and challenges in microbial organic acids production are discussed.

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Introduction

Since ancient times microbial fermentations have been traditionally used in food and feed applications, such as for production of organic acids, alcohols, amino acids and vitamins. The introduction of citric acid production via microbial fermentation around 1920 marks the foundation of industrial-scale microbial fermentation for chemicals production. This was further cemented with the introduction of first penicillin production and later production of many other antibiotics, and with the introduction of industrial fermentations for the production of enzymes in the 1970s. With the emergence of genetic engineering it became possible to produce compounds that are not native to microbes and enabled construction of microbial cell factories that can be used for the production of chemicals through metabolic engineering [1,2]. Further developments in the field of systems and synthetic biology, along with evolution and selection has allowed great advances in our engineering capability to develop highly efficient microorganisms that can produce chemicals of interest [3,4].

With the desire to develop more sustainable processes for production of fuels, chemicals and materials, the chemical industry has started to exploit these technologies to develop novel bioprocesses for production of both fine and commodity chemicals. Organic acids constitute a key group among top platform chemicals that can be produced by microbial fermentation. The current market for microbial organic acid production is still moderate and often associated with food applications. However, one can envision its future market potential with this transition towards their use as building blocks for synthesis of a wide range of chemicals. Here, we review recent advances in metabolic engineering to develop microbial cell factories for the production of industrially important organic acids, including citric acid and lactic acid as examples of industrially large-scale implemented production of organic acids, and succinic acid and 3-hydroxypropionic acid as representations at the stage of commercialization, with a particular emphasis on the latest concerning product yield, productivity and achieved product concentrations. Also, we discuss the key limitations and challenges in the field of microbial organic acid production.

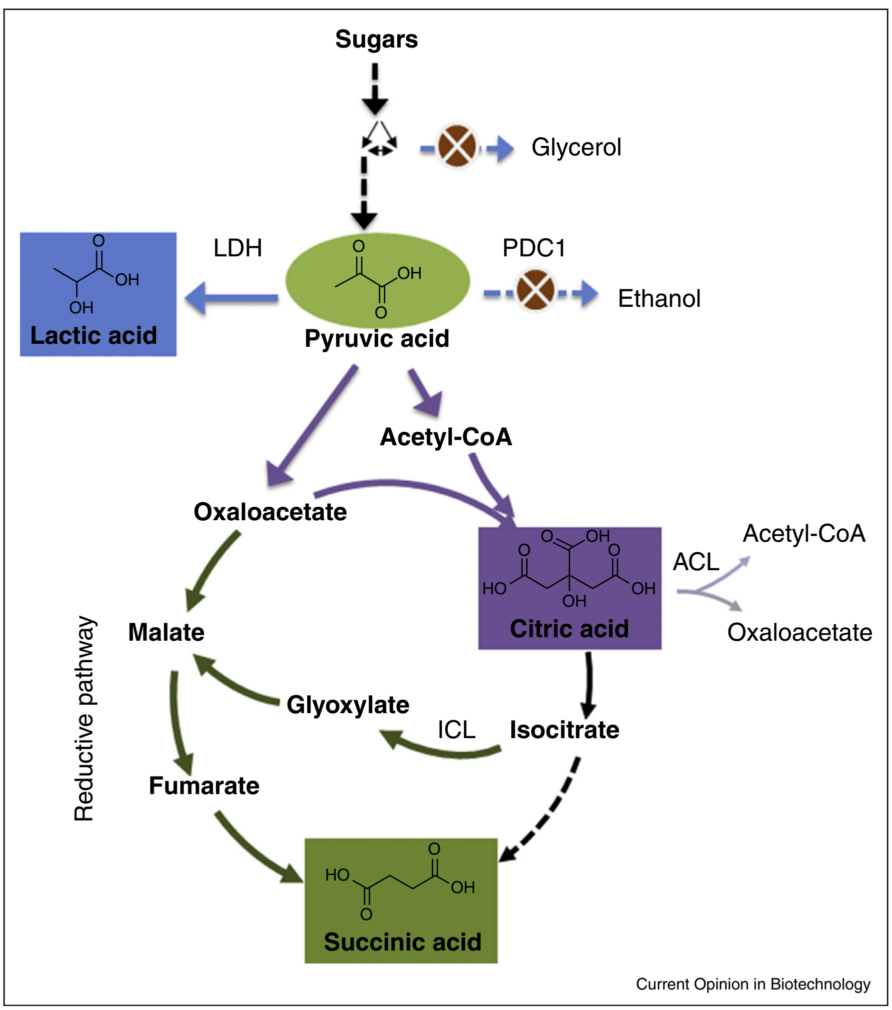
Citric acid

Citric acid is a successful example of a commercially bio-produced organic acid with annual production estimated at 1.75 million tons in 2011 and having a continuous increasing demand/consumption [5]. Besides its predominant use in the food and beverage industry, citric acid has been also used in pharmaceuticals and a range of technical and industrial segments as well. The current industrial production relies on *Aspergillus niger* through submerged fermentation of starch-based or sucrose-based media [6]. Industry is seeking newer cheap and economic process technologies to enhance the competitiveness to cope with the current adverse market conditions. A cost reduction, in this light, can be achieved by shifting towards using less expensive substrates such as agro-industrial wastes and their by-products [7]. However, agro-industrial wastes are often complex, which may require pretreatment to ensure proper nutrient availability and rheological properties, and this may further complicate the downstream processing. Furthermore, for production of chemicals to be used for polymer production, where a high purity of the chemical is required, the use of complex substrates may increase the costs of purification. Alternatively, the use of yeast for instance *Yarrowia lipolytica* has also been developed for the production of citric acid from various carbon sources, for example, inulin through surface displaying an exo-inulinase [8]. Further engineering the strain by down-regulation of the ATP-citrate lyase (ACL, Figure 1) and enhancing the expression of isocitrate lyase (ICL, Figure 1) has achieved citric acid production at a titer of 84 g/L from 10% inulin [9]. Despite this impressive titer, it is still significantly lower than what has been achieved using *A. niger* grown on glucose, that is, a yield of 0.88 g/g glucose and a titer of 240 g/L [10], but it demonstrated that metabolic engineering offers a potentially useful approach for improving yields and fermentation rates in *Y. lipolytica*.

Lactic acid

Lactic acid has traditionally been produced through fermentation and used widely in food, cosmetic, pharmaceutical and leather industries. The main current application of lactic acid is however as a chemical intermediate for the production of polylactic acid, which is one of the most promising biodegradable polymers. The global annual production of lactic acid has been estimated at 370,000 tons in 2011, ranking among the high-volume products being biologically produced [11]. Biological production of lactic acid was traditionally carried out using lactic acid bacteria, but this process is not economical viable for large industrial-scale fermentations owing to the requirements of nutritionally rich media and moderate pH conditions [12]. Other natural producers such as *Rhizopus oryzae* have been exploited for lactic acid production and a high titer of 231 g/L of lactic acid with yield

Figure 1



Overview of the metabolic pathways for production of lactic acid, citric acid and succinic acid from sugars. ACL, ATP-citrate lyase; ICL, isocitrate lyase; LDH, lactate dehydrogenase; PDC1, pyruvate decarboxylase 1.